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# Isotopic variability of δ¹³C and δ¹⁵N signals in cave systems: insights from the blind tetra *Astyanax mexicanus*

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## Abstract

Stable isotope analysis allows the study of element cycles in ecosystems and trophic ecology. δ¹³C reflects the diversity of primary productivity, while δ¹⁵N is a good indicator of trophic levels of organisms. Caves have limited resources due to the absence of light, reducing the trophic chains in these ecosystems. These extreme conditions impose strong selection pressures on cave-dwelling organisms, known as troglobites, which exhibit specific adaptations such as vision and pigment loss, and metabolic and physiological differences with their surface counterparts. The species *Astyanax mexicanus* corresponds to a model organism in the study of regressive evolution, which presents two different ecotypes, a widely distributed surface morph, and a cave-dwelling morph present in at least 34 caves in three karst regions of San Luis Potosí and Tamaulipas, Mexico. In the present study, we characterized the δ¹³C and δ¹⁵N of seven cave populations of *A. mexicanus*, corresponding to two karst regions: Sierra de El Abra and Sierra de Guatemala, representing distinct genetic cavefish lineages. We also developed a Nutrient Input Index (NI), to assess whether cave geomorphology influences resource availability. We found isotopic differences between caves and regions analyzed. Caves in the Sierra de El Abra showed higher δ¹⁵N values and wider trophic niche ranges compared with those in the Sierra de Guatemala, reflecting a more complex trophic network tentatively

associated also with its geological history. In addition, a relationship was observed between the proximity of pools to the surface and the $\delta^{13}C$ values, which could suggest differences in NI directly associated with cave geomorphology, impacting selective forces across the different cave systems.

## Introduction

Caves are formed by different types of rocks and through various geological processes, with dissolution as the main forming agent, and limestone and dolomite as the substrates of the largest and most prevalent formations (Moldovan et al. 2018; Culver and Pipan 2019a). They represent distinctive ecosystems characterized by their physical structure and biological functioning (Moldovan et al. 2018). The geological setting, external terrestrial conditions including vegetation type, fauna, and soil, as well as hydrological features such as erosion, water flow, and material transport, delineate the cave ecosystem's boundaries, define its diverse habitats, and regulate the movement of energy and matter within the systems (Rouch 1987; Simon 2019).

Water bodies within caves exhibit variations in temperature, flow rate, and chemical composition depending on their connection to the surface. The terrestrial environment of caves can be classified into five zones: entrance, twilight, transition, deep zone, and stagnant air (White and Culver 2019), illustrating a gradient from the entrance towards deeper regions where light diminishes and temperature and humidity stabilize (Howarth 1983). Consequently, the terrestrial and aquatic habitats within caves should not be perceived as discrete systems but rather as a continuum characterized by relatively constrained energy flow (Poulson and Lavoie 2000).

These systems are characterized by perpetual darkness and oligotrophy, resulting from the limited availability of organic matter and low energy density (Moldovan et al. 2018). Consequently, the trophic structure of caves is considered less complex in terms of energy and nutrient dynamics compared to the surface environments (Moldovan et al. 2018), the latter playing a significant role in sustaining cave ecosystems (Kováč 2018). This connectivity is notably pronounced in limestone formations, where water percolation is a key characteristic (Dunne et al. 2002; Romero 2009; Kováč 2018). The combination of cave zones and available nutrient sources is important for species adaptation, imposing strong selective pressures for their evolution (Howarth and Moldovan 2018; Culver and Pipan 2019b).

In cave ecosystems, two distinct trophic networks have been described: detritus-based systems, which rely on decaying plant or animal matter, and chemoautotrophic systems, which are supported by bacteria capable of converting inorganic carbon (such as carbon dioxide or bicarbonate) into organic compounds (Venarsky and Huntsman 2018). Detritus-based cave ecosystems primarily operate through classical photosynthetic pathways, wherein organic material is transported by water flow from the surface, and also percolation, root growth inwards, and active or passive transport by animals (Simon et al. 2003; Culver and Pipan 2019b; Fong 2019; Simon 2019). In contrast,

chemoautotrophy-based cave ecosystems are sustained by bacteria that derive energy from the oxidation of inorganic compounds, notably sulfur (the most common) and methane. These bacteria transform carbon dioxide or bicarbonate into microbial biomass, facilitating primary productivity, particularly in the deepest zones of large caves (Sarbu et al. 1996; Venarsky and Huntsman 2018; Engel 2019).

Stable isotope ratio analysis (SIRA) stands as a valuable tool utilized to study element cycles in ecosystems, and trophic dynamics, particularly in elucidating the trophic positions of species within food webs (Boecklen et al. 2011; Brand and Coplen 2012). Specifically, the isotopic signatures of carbon ($\delta^{13}$C) and nitrogen ($\delta^{15}$N) provide insights into the organic carbon sources and trophic levels, respectively (Peterson and Fry 1987). This analytical approach has greatly advanced our understanding of trophic interactions and ecological roles in aquatic ecosystems(Vander-Zanden and Rasmussen 1999; Pilger et al. 2010; Cucherousset et al. 2012; Ornelas-García et al. 2018). For instance, $\delta^{13}$C signals indicate the horizontal size of a food web (reflecting the diversity of primary producers) while $\delta^{15}$N signals represent the number of vertical levels, where primary and secondary consumers are located (Bearhop et al. 2004; Boecklen et al. 2011). Additionally, stable isotopes facilitate the assessment of species' trophic niche areas (Córdova-Tapia and Zambrano 2016), which represents the extent of diversity in a food chain and an approximation of the variety and abundance of nutrients (Bearhop et al. 2004; Jackson et al. 2011). One of the most prominent methods for studying these isotopic signals is the so-called "Layman metrics", which comprise a suite of six measures delineating the structure, size of the ecological niche, and various trophic levels present within food webs (Layman and Post 2008).

In Mexico, one of the most studied cave-dwelling species is the Mexican tetra, *Astyanax mexicanus*, which has two morphs: a surface ecotype widely distributed in northern Mexico and the southern United States (Ornelas-García et al. 2008; Ornelas-García and Pedraza-Lara 2016), and a cave ecotype known from at least 34 distinct cave populations distributed in three karstic regions, between the states of San Luis Potosí and Tamaulipas: Sierra de La Colmena, Sierra de El Abra, and Sierra de Guatemala (Elliott 2018; Miranda-Gamboa et al. 2023). One unique aspect of this organism is that its cave populations evolved through at least three independent events (Garduño-Sánchez et al. 2023), providing a valuable opportunity to study parallel regressive evolution (Hernández-Lozano et al. 2024). The cave ecotype exhibits troglobitic traits, such as loss of vision and pigmentation (Jeffery 2009; McGaugh et al. 2014), as well as some features related to complex traits, including reduced sleep (Keene and Duboué 2018; Keene et al. 2024) and reduced aggressive behavior in certain populations (Elipot et al. 2013). One of the most notable adaptations in these cave populations is their ability to tolerate prolonged periods of starvation, which drives both metabolic and behavioral adaptations for resource acquisition in cave environments (Borowsky 2018; Pozo-Morales et al. 2024). Despite significant evidence for nutrient scarcity in these habitats, little is known about the trophic ecology, particularly nutrient availability, and whether these nutrient constraints differ among cave populations.

In this study, Stable Isotope Ratio Analysis (SIRA) is employed to investigate the trophic ecology of seven cavefish populations of *A. mexicanus* across two karstic geo-

graphic regions: the Sierra de El Abra and the Sierra de Guatemala. The primary goals of this research are: 1) to discern differences in trophic ecology among distinct populations of *A. mexicanus* inhabiting seven caves; 2) to determine differences between two cave systems, each representing a distinct lineage, considering their evolutionary backgrounds; and 3) to develop an index for nutrient influx based on cave geomorphology and evaluate its correlation with isotopic values to explore the potential impact of cave geomorphology on resource availability. This study represents a pioneering effort in elucidating the trophic structure of cavefish in Mexico.

## Methods

### Study Area

The Sierra de El Abra and Sierra de Guatemala systems are karst mountains in northeastern Mexico along the eastern margin of the Sierra Madre Oriental (Fig. 1). The Sierra de El Abra system is located within the Biosphere Reserve Sierra de El Abra-Tanchipa San Luis Potosí and Tamaulipas, while the Sierra de Guatemala system is situated in the Biosphere Reserve El Cielo, Tamaulipas. These systems are characterized by their highly cavernous nature and are home to one of the world's highest
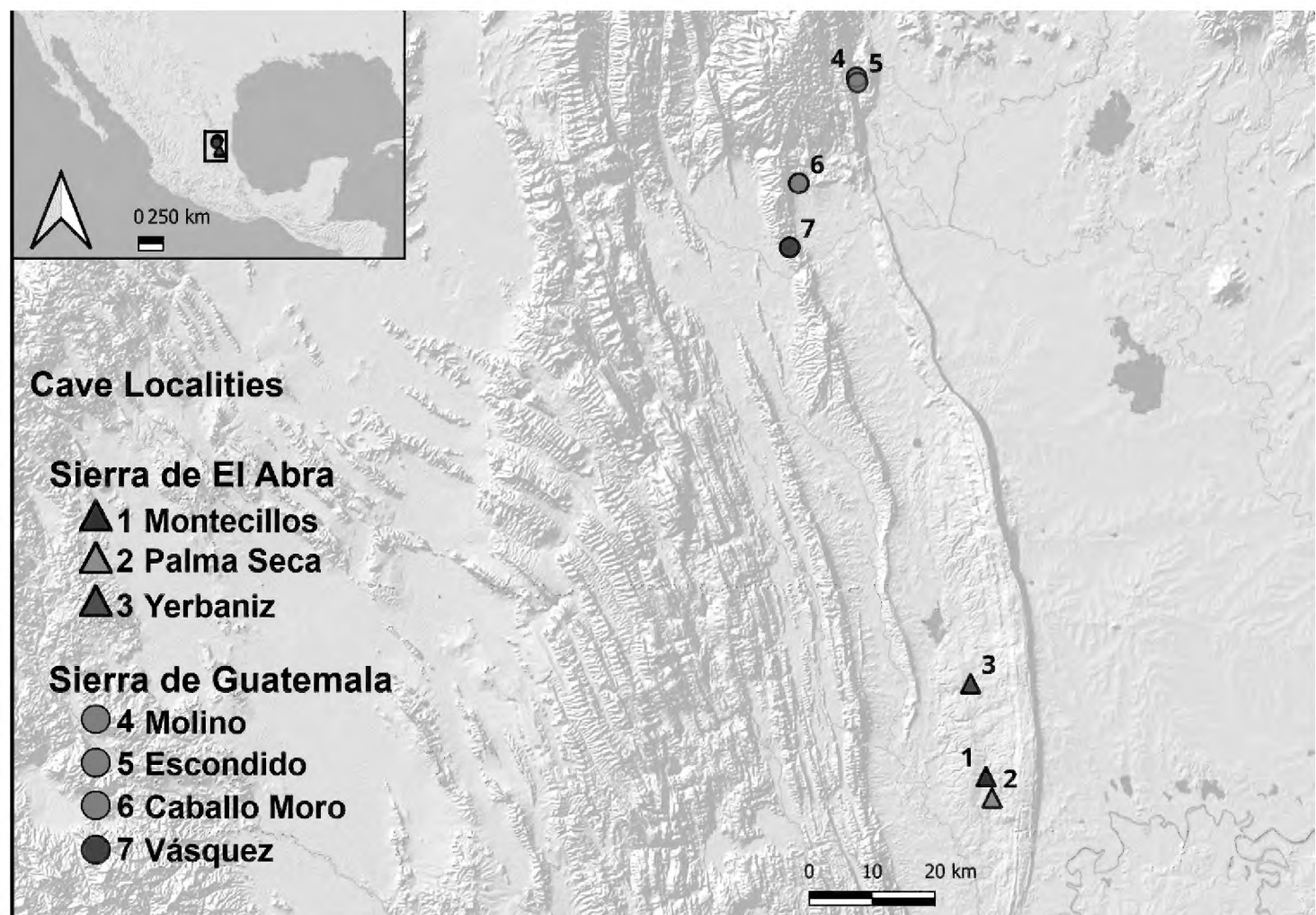


**Figure 1.** Geographic location of the sampled caves. Triangles represent caves in Sierra de El Abra system. Circles represent caves in the Sierra de Guatemala system.

flowing karst springs (Gary and Sharp 2006). There are currently 30 known populations of *A. mexicanus* in both regions (Elliott 2018; Miranda-Gamboa et al. 2023).

## Sampling

Sampling was conducted between January and May, 2017, corresponding to the dry season. A total of 47 fish samples were collected from seven caves. In the Sierra de El Abra system, fish were sampled from Montecillos (n = 3), Palma Seca (n = 13), and Yerbaniz (n = 5), while in the Sierra de Guatemala system, fish were sampled from Caballo Moro (n = 5), Escondido (n = 8), Molino (n = 8), and Vásquez (n = 5) (Fig. 1). Fish were captured using hand nets, then photographed, weighed, sexed, and euthanized to obtain muscle tissue for stable isotope analysis. All sampling procedures were conducted under permit SGPA/DGVS/02438/16, issued by the Mexican Authority (SEMARNAT). All fish were treated following the Official Mexican Standards for the humane treatment of animals during collection (NOM-051-ZOO-1995).

## Stable isotopes

Muscle tissues of fish were dehydrated by placing them in salt (NaCl) for transportation and then frozen at -80 °C until processing. To remove salt residues, the samples were hydrated in milliQ water, and then dried at 60 °C for 48 hours. Once the samples were dry, they were ground into a fine powder and stored in sterile 2 mL tubes for further analysis. The samples were analyzed for their carbon and nitrogen signature using continuous flow isotope ratio mass spectrometry at the Center of Stable Isotopes at the University of New Mexico. The mean standard deviation between the samples and laboratory standards was 0.04‰ for $\delta^{13}C$ and 0.07‰ for $\delta^{15}N$.

## Nutrient input index

Previous studies have estimated some geomorphological features of the caves used in this study, such as depth and entrance elevation in order to estimate the cave's age (Espinasa and Espinasa 2015; Elliot 2018). Following this, we developed the nutrient input index (NI) to estimate the influx of allochthonous material into subterranean pools, based on the geomorphological characteristics of caves, such as the proximity of pools to the surface, cave depth and phreatic level:

$$NI = \frac{DEP}{BLC - DC}$$

Where NI is the Nutrient Input Index; DEP is the distance from the cave entrance to the first fish pool; BLC is the local base level of the cave, which represents the minimum elevation of the local phreatic mantle (groundwater level determined by

local geology), estimated in relation to the minimum elevation of the nearest rivers (Goudie 2003); and DC is the cave's depth. The latter two geomorphological characteristics were obtained from published literature: BLC was sourced from Espinasa and Espinasa (2015), and DC from Elliot (2018). For the DEP, seven topographic cave maps were downloaded from data provided by the Association for Mexican Cave Studies (AMCS 2022, http://www.mexicancaves.org/, accessed in 2022). The total distance traversed in both vertical and horizontal directions within the cave passages was measured using the imageJ v.1.54 software (Schneider et al. 2012), calibrated with the assigned scale for each map (Suppl. material 1). The NI index compares the two distance values, DEP and BLC-DC. If the calculated value is greater than 1, it indicates that the pool is closer to the entrance, resulting in a higher input of allochthonous material. Conversely, values less than 1 indicate that the pool is farther from the entrance and closer to the bottom of the cave, where the influence of autochthonous material is greater.

## Statistical analysis

To investigate differences in the $\delta^{15}N$ and $\delta^{13}C$ signals between Sierra de El Abra and Sierra de Guatemala, a Kruskal-Wallis analysis of variance was conducted. Subsequently, pairwise comparisons were made using the Mann-Whitney U test to examine differences at the population level, with significance values corrected by a Bonferroni procedure (Dunn 1961). To explore the relationship between the NI and isotopic signals, we conducted linear regressions using the NI values for the different caves and the average values of the $\delta^{13}C$ and $\delta^{15}N$ isotopic signals. All statistical analyses were performed using the R software (R Core Team 2023).

Layman's metrics were used to conduct a trophic niche analysis, which included: nitrogen range (NR), carbon range (CR), total niche area (TA), mean distance to centroid (CD), mean distance to nearest neighbor (NND), and standard deviation of nearest neighbor distance (SDNND) (Layman and Post 2008). NR is the interval between the organism with the highest and lowest values of $\delta^{15}N$. It describes the population's ability to feed on items from different trophic levels. CR is the interval between the organism with the highest and lowest values of $\delta^{13}C$. It describes the population's ability to feed on items from different producers at the base of the food web. TA represents the total trophic niche area of the population and is related to feeding diversity. CD measures the average degree of diversity within the population based on the spacing between organisms. NND measures the overall density of organism clustering, with lower values suggesting greater trophic redundancy. SDNND is less affected by sample size than NND and suggests a more uniform distribution of trophic niches (Jackson et al. 2001; Layman and Post 2008). In addition, a Bayesian trophic niche analysis was conducted using standard ellipse areas (SEA) to account for the potential effect of sample size, following the methodology of Jackson et al. (2011). Two Markov chain Monte Carlo (MCMC) simulations were run with 10,000 permutations each, and the first 1,000 values were discarded as burn-in.

## Results

The analysis of variance revealed significant statistical differences for both stable isotopes among populations ($\delta^{15}$N: H = 25.74, p < 0.01; $\delta^{13}$C: H = 15.34, p = 0.01). At the population level, Yerbaniz cave had the lowest $\delta^{15}$N value within the Sierra de El Abra system, while Montecillos had the highest $\delta^{15}$N value (9.2‰ ± 0.7 and 10.9‰ ± 0.6, respectively), making it the most $\delta^{15}$N enriched population between the two geographic regions. Among the caves in the Sierra de El Abra, significant differences were found between Yerbaniz and Montecillos caves (U = 0, p < 0.05) and between Yerbaniz and Palma Seca (U = 5.5, p = 0.01) (Table 1). Within the Guatemala system, Molino cave had the lowest $\delta^{15}$N value (8.6‰ ± 0.4), with significant differences observed between Molino and all others caves in the Sierra Guatemala: Caballo Moro (U = 0, p < 0.01), Vásquez (U = 0, p < 0.01), and Escondido (U = 7.5, p = 0.01). When comparing between the Sierra de El Abra and Sierra de Guatemala systems, we observed significant differences between Montecillos from Sierra de El Abra and the other two caves from Sierra de Guatemala: Molino (U = 0, p = 0.01) and Vásquez (U = 0.5, p < 0.05). We also recovered differences between Palma Seca from Sierra de El Abra and Molino from Sierra de Guatemala (U = 1, p < 0.01). The above suggests a link between isotopic variation and lineages associated with geographic systems.

Montecillos had the lowest $\delta^{13}$C value across both geographic regions (-29.8‰ ± 2.4). Within the Sierra de El Abra system, significant differences were found between the Montecillos and the Palma Seca caves (U = 2, p < 0.05). In the Sierra de Guatemala system, significant differences were observed between Molino and Escondido caves (U = 11.5, p < 0.05). Between systems, differences were found between Montecillos and Caballo Moro caves (U = 0, p < 0.05), as well as between Montecillos and Escondido (U = 0, p = 0.01).

The Layman metrics showed that the trophic niche area (TA) in the Sierra de El Abra region was broader and exhibited lower trophic redundancy (CD, NND, and SDNND) compared to the Sierra de Guatemala region (Table 2). At the population level, Palma Seca cave has the widest niche amplitude in both geographic systems (7.06), whereas Vásquez has the narrowest niche (0.42). In general, the populations

**Table 1.** Statistical differences in $\delta^{13}$C and $\delta^{15}$N among sites and systems. The p values obtained from paired comparisons between caves are shown. Values with * are statistically significant.

| | $\delta^{13}$C | Guatemala | | | | El Abra | | |
| | $\delta^{15}$N | Molino | Caballo Moro | Vásquez | Escondido | Montecillos | Palma Seca | Yerbaniz |
|---|---|---|---|---|---|---|---|---|
| Sierra de Guatemala | Molino | | 0.88 | 0.99 | <0.05* | 0.19 | 0.92 | 1 |
| | Caballo Moro | <0.01* | | 0.99 | 0.69 | <0.05* | 0.99 | 0.9 |
| | Vásquez | <0.01* | 1 | | 0.26 | 0.13 | 0.99 | 0.99 |
| | Escondido | 0.01* | 0.76 | 0.60 | | 0.01* | 0.21 | 0.09 |
| Sierra de El Abra | Montecillos | 0.01* | 0.07 | <0.05* | 0.12 | | <0.05* | 0.29 |
| | Palma Seca | 0.01* | 0.16 | 0.08 | 0.30 | 0.31 | | 0.95 |
| | Yerbaniz | 0.07 | 0.11 | 0.14 | 0.10 | <0.05* | 0.01* | |

**Table 2.** Layman metrics for each population and system. NR: nitrogen range, CR: carbon range, TA: total niche area, CD: mean distance to centroid, NND: mean distance to nearest neighbor, SDNND: standard deviation of nearest neighbor distance, $\delta^{15}N$: mean isotopic signature of nitrogen, $\delta^{13}C$: mean isotopic signatures of carbon, NI: nutrient input index.

| | Trophic niche analysis | | | | | | Nutrient index | | |
|---|---|---|---|---|---|---|---|---|---|
| | NR | CR | TA | CD | NND | SDNND | $\delta^{15}N$ | $\delta^{13}C$ | NI |
| **Sierra de El Abra** | **1.73** | **3.4** | **3.27** | **1.57** | **2.01** | **0.95** | | | |
| Montecillos | 1.2 | 4.2 | 2.49 | **1.93** | **2.18** | **1.69** | 10.9 | -29.8 | 60 |
| Palma Seca | **2.5** | **6.1** | **7.06** | 1.62 | 0.74 | 0.42 | 9.1 | -27.23 | 67 |
| Yerbaniz | 1.6 | 2.4 | 1.79 | 0.95 | 0.99 | 0.09 | 10.4 | -26.43 | -4.4 |
| **Sierra de Guatemala** | **1.45** | **2.5** | **1.41** | **1.01** | **1.05** | **0.45** | | | |
| Caballo Moro | 1.3 | 2 | 0.9 | 0.72 | 0.69 | 0.29 | 9.97 | -26.11 | 1.2 |
| Escondido | **3.2** | **5.9** | **6.53** | **1.7** | **1.03** | **0.7** | 8.55 | -27.22 | -6.2 |
| Molino | 1.1 | 4 | 2.29 | 1.3 | 0.45 | 0.17 | 9.98 | -27.7 | 20 |
| Vásquez | 0.6 | 1.4 | 0.42 | 0.48 | 0.43 | 0.22 | 9.94 | -26.76 | -4.1 |

showed overlap in their trophic niche areas; however, Montecillos did not overlap with any other populations (Fig. 2A). The NI index showed that populations living in deeper caves (i.e., Vásquez) have negative index values (Table 2). Escondido cave has the lowest value (NI=-6.2), followed by Yerbaniz (NI=-4.4), and Vásquez (NI=-4.1). In contrast, populations with pools closer to the cave entrances have higher NI index values, such as Montecillos (NI = 60) and Palma Seca (NI = 67).

Taken together, the Bayesian Standard Ellipse Area (SEA$_B$) and Layman's analyses showed differences between populations (Fig. 2B) and regions (Suppl. material 2). At the population level, Montecillos showed the highest values for total niche area based on SEA$_B$, which were consistent with other metrics such as mean distance to centroid (CD), mean distance to nearest neighbour (NND) and standard deviation of nearest neighbour distance (SDNND). While in Layman's metrics the highest values for niche area (TA) were found at Palma Seca. Vásquez showed the lowest SEA$_B$ values, consistent with Layman's analyses. When comparing the two regions, the Sierra de El Abra system has a wider niche area (TA) and lower trophic redundancy (CD, NND, and SDNND) than the Sierra de Guatemala region.

Contrasting relationships were observed between the isotopic signals of $\delta^{15}N$ and $\delta^{13}C$, and the NI index. For nitrogen, the analysis indicated a trend where nitrogen values increased as the index values rose (Fig. 3A); however, this relationship was not statistically significant. In contrast, a significant negative relationship was observed for the carbon signal (Fig. 3B).

## Discussion

Stable isotope analyses of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) provide useful information for understanding the trophic structure of ecosystems, as they provide insights into trophic positions and food sources (Post 2002). This study represents
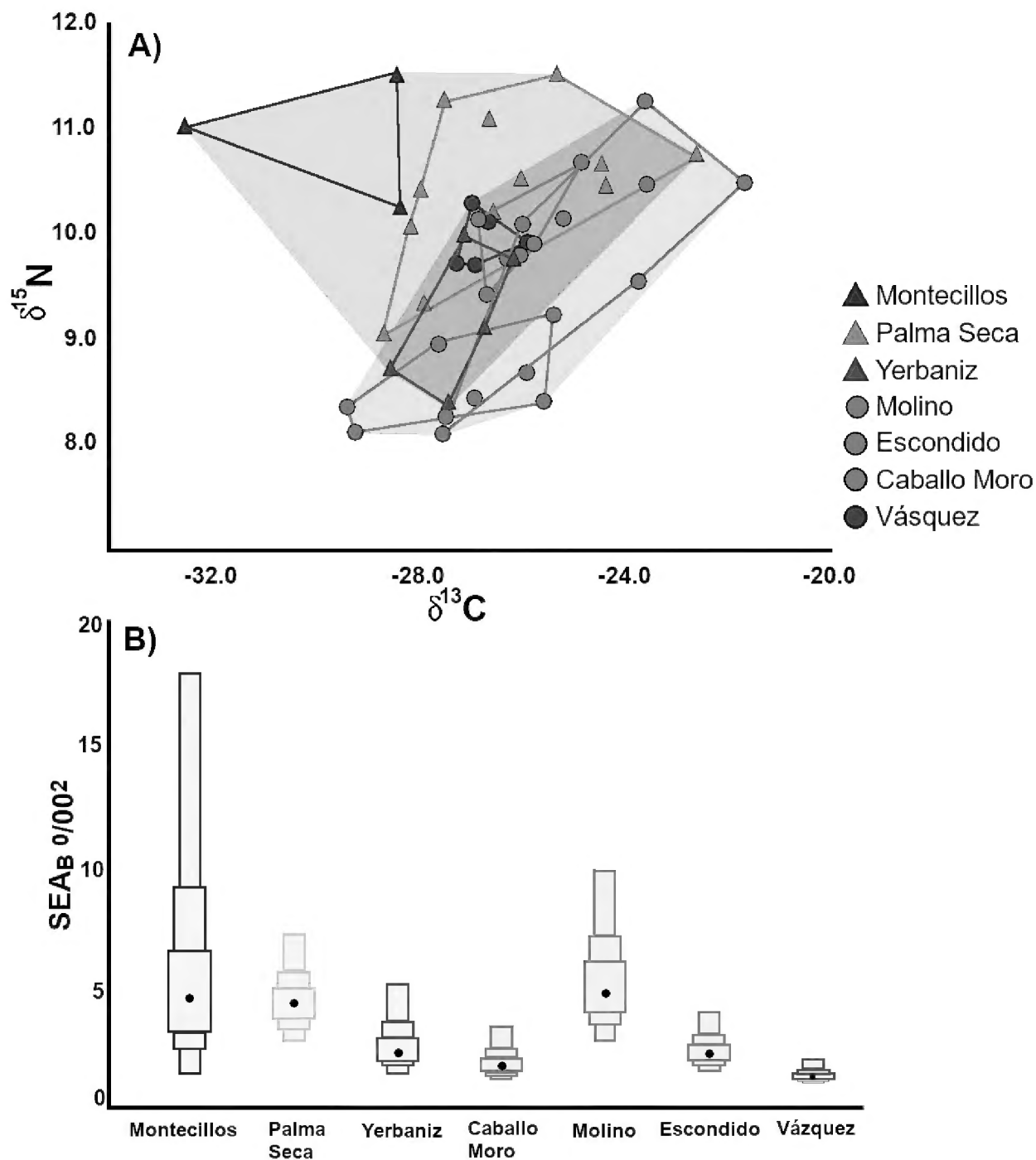
**Figure 2.** Trophic niche analysis of isotopic signals **A** bi-plot of $\delta^{15}N$ and $\delta^{13}C$: triangles represent populations from the Sierra El Abra, enclosed by the blue polygon, while circles represent populations from the Sierra de Guatemala, enclosed by the red polygon **B** standard ellipse area (SEA$_B$) of the total niche area for each population. The black dot indicates the mean. Populations from the Sierra El Abra are depicted in blue tones, while those from the Sierra de Guatemala are depicted in red tones.

the first attempt to understand the trophic ecology of two independent lineages of cave populations of *A. mexicanus*. We found differences in the isotopic signals among regions and populations. Overall, the Sierra de El Abra region displayed a broader trophic niche area and lower trophic redundancy, with a relationship between cave geomorphology and isotopic values.

Comparing our regions, there is a 2.3‰ $\delta^{15}N$ difference between the most enriched population (Montecillos) and the least enriched population (Molino). The $\delta^{15}N$ helps explain the vertical complexity of the trophic network by identifying the trophic levels formed by producers and predators (Layman and Post 2008; Jackson et al. 2011). It has been observed that a difference of 3‰ - 4‰ in $\delta^{15}N$ typically exists between trophic

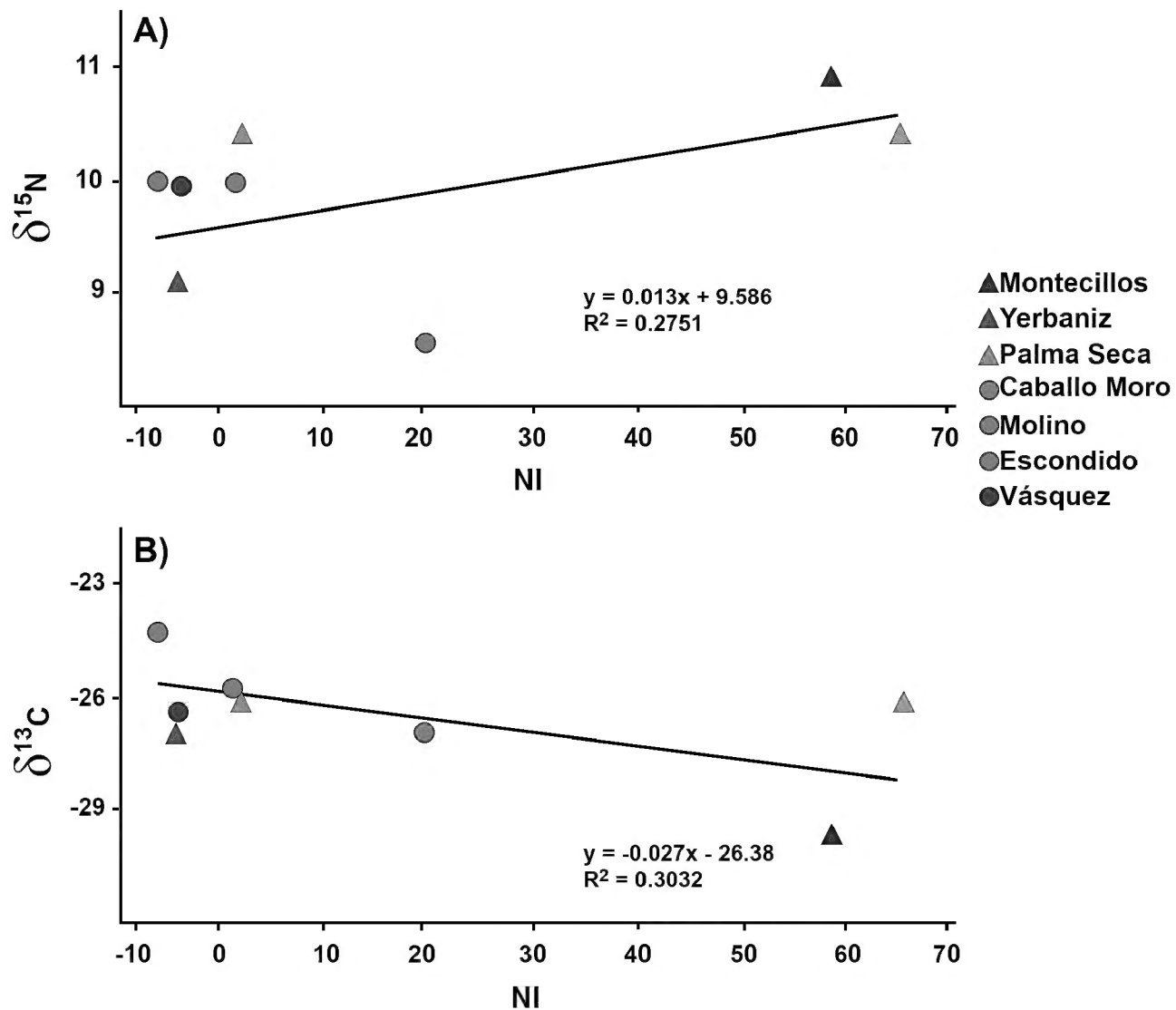**Figure 3.** Linear regression of isotopic signals δ¹⁵N (**A**) and δ¹³C (**B**) against the nutrient input index (NI). Triangles represent populations from the Abra system, while circles represent populations from the Guatemala system.

levels (Vander-Zanden and Rasmussen 1999; Post 2002), suggesting that cavefish trophic levels may be similar in different caves. However, the isotopic signal of a consumer alone is generally insufficient to accurately infer trophic position without an appropriate isotopic baseline, typically comprising primary producers and primary consumers (potential prey) (Vander-Zanden and Rasmussen 1999; Post 2002). Differences in $\delta^{15}N$ and $\delta^{13}C$ among caves within the same region may reflect variations in the trophic resource availability, local ecological processes such as differences in trophic networks or environmental conditions in each cave, and the differential use of habitats or resources by local populations (Wilson et al. 2021). On the other hand, the differences observed between the Sierra de El Abra and Sierra de Guatemala regions could be linked to divergence processes among lineages driven by evolutionary history, geographic isolation, or ecological landscape differences that affect resource availability and trophic interactions. Together, these findings suggest that local ecological processes, such as resource availability and the structure of trophic networks, can be as influential as geographic or evolutionary factors in driving the observed isotopic variation.

Previous studies have reported that *A. mexicanus* cavefish primarily feed on detritus in pools but are also active predators, particularly during the early stages of

their life when they mainly consume microcrustaceans, isopods, and arthropods (Espinasa et al. 2017). It is suggested that active predation during the juvenile stage allows them to feed adequately until adulthood (Espinasa et al. 2017). However, during multiple expeditions across a year, these fish have also been observed to be opportunistic feeders, consuming animals that fall into the pools, such as arthropods, fish, and detritus, which could explain the variation in $\delta^{15}N$ among populations (Wilson et al. 2021). Moreover, studies of the stomach microbiome in cave populations have suggested that these cavefish may exhibit omnivorous habits (Ornelas-García et al. 2018). Other cave vertebrates like the cave salamander *Eurycea spelaea* feed on bat guano, providing nutritional value comparable to potential invertebrate prey (Fenolio et al. 2006), for the *A. mexicanus*, bat guano has been suggested as a food source (Espinasa et al. 2017). Therefore, while our findings are insightful, future studies should consider examining the entire trophic network or corroborating isotopic signals with stomach content analysis.

The $\delta^{13}C$ signal helps us understand the diversity of carbon sources within the trophic network (Bearhop et al. 2004; Layman and Post 2008; Jackson et al. 2011). Our results showed slight variations in the $\delta^{13}C$ signal, ranging from -29.8‰ in Montecillos to -24.7‰ in Escondido. These values are similar to those reported for plant material in other anchialine karst systems ($\delta^{13}C$ = -35‰ to -25‰, Brankovits et al. 2017), but differ from those associated with methanotrophic bacteria ($\delta^{13}C$ = -75‰ to -45‰; Brankovits et al. 2017; Chavéz-Solis et al. 2020). Based on this, we can infer that the primary source of carbon in these caves is plant material originated from the surface, even in the deepest caves such as Vásquez ($\delta^{13}C$ = -26.76‰, depth = 270 m). In these cases, the decomposing plant material may be part of the fish diet or the diet of primary consumers, which in turn could be prey for *Astyanax* cavefish. That said, we acknowledge that other primary sources of carbon may exist for subterranean foodwebs (e.g., DOC and POC percolating from the surface, and microbial biomass) (Pacioglu et al. 2023) which could be contributing to observed *Astyanax* isotopic values. Given the nature of our sampling scheme, we were not able to isolate individual sources. It would be interesting to separate these sources and contrast their importance among cave systems; such effort will require a much different sampling approach to what we were able to apply in our investigation. Additionally, it is important to consider that our results could be affected by seasonality, as has been reported in previous trophic studies in *Astyanax* cavefish (Wilson et al. 2021).

Trophic niche analysis based on Layman's metrics revealed that the Sierra de El Abra had a broader total niche area and lower trophic redundancy compared to the Sierra de Guatemala system. This metric is commonly used to quantify the ecological niche occupied by a population and is related to trophic diversity (Bearhop et al. 2004; Layman and Post 2008; Jackson et al. 2011). According to our results, the Palma Seca cave population in the Sierra de El Abra system had the highest total niche area (6.10), in contrast to the Vásquez population in the Sierra de Guatemala system, which had the lowest value (0.42) and is the deepest cave of the study. Moreover, Vásquez also exhibited the lowest standard deviation in the trophic niches values among individu-

als (SDNND = 0.22), which indicates higher trophic redundancy and, consequently, more intense competition for resources within the population (Bearhop et al. 2004; Layman and Post 2008; Jackson et al. 2011). This can be explained by the fact that in deeper caves (DC), it is more difficult for allochthonous material to reach the pools.

The negative and significant relationship between $\delta^{13}C$ and the NI index is consistent with the idea that the deeper the cave, the more difficult it is for plant sources to enter from the surface. Although no significant relationship was found for $\delta^{15}N$, the trend suggests that caves with shallower depths and shorter distances from the basin entrance may support more complex trophic networks. Habitats close to the entrance of caves, where access to resources is easier, typically have higher biodiversity (Simões et al. 2015). Larger caves have greater zonation and may also exhibit high diversity, with different organisms highly specialized for troglobitic life inhabiting each zone (Ferreira 2000; Souza-Silva et al. 2011; Simões et al. 2015; Culver and Pipan 2019b).

Based on these observations, we propose that the influx of allochthonous material significantly influences the trophic ecology dynamics within each cave. This influx is driven by factors such as the transport of plant material by water currents, rainwater percolation, and the ease with which fauna can access the cave (White and Culver 2019). This is reflected in the NI index obtained, with positive NI index values in Montecillos and Palma Seca in the Sierra de El Abra system, and Molino and Caballo Moro in the Sierra de Guatemala system. Conversely, caves with negative NI index values, such as Yerbaniz, Escondido, and Vásquez, differ on attributes such as size, distance from the entrance to the pool, and depth. Additionally, nutrient input may come directly from groundwater. In this sense, the NI index suggested in the present study represents a pioneer metric to estimate the trophic ecology in the cave systems, thus, this index can be useful for indirectly describing the trophic ecology of cave systems. To corroborate this, it would be necessary to conduct a more exhaustive study of the trophic networks, incorporating direct measurements of resource inputs and analyses of other cave systems. However, we consider this a promising approach to infer the intensity of selective pressures on the organisms inhabiting these systems, based on the geomorphology of karst systems as a whole.

Additionally, both geographic regions have complex hydrological patterns that could allow interconnection between different caves (Elliott 2018). These patterns may also be influenced by seasonal changes, such as the rainy season, when the water levels of external aquatic bodies rise, as reported for other tropical caves (Souza-Silva et al. 2011; Simões et al. 2015). Furthermore, in the future we could evaluate the season differences in organic matter and its influence in the trophic ecology variation of cavefish of *A. mexicanus.*

## Conclusion

The isotopic signals of $\delta^{15}N$ and $\delta^{13}C$ revealed differences among the analyzed caves, suggesting that each cave may have distinct nutrient cycle dynamics. Layman's metrics indicated that populations in the Sierra de El Abra system occupy a larger trophic niche area with lower trophic redundancy compared to those in Sierra de Guatemala system.

Despite variations in $\delta^{15}N$ values, our results suggest that the trophic level among the *Astyanax* cavefish populations is the same. Additionally, the isotopic signals appear to be influenced by geomorphological characteristics of caves, such as depth and distance from the entrance to the pool, which may directly affect nutrient input in each cave.

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## Supplementary material 1

### Supporting data

Authors: Jorge Hernández-Lozano, Fernando Córdova-Tapia, Ramses Miranda-Gamboa, María de Lourdes Vázquez-Cruz, Carlos Garita-Alvarado, Norman Mercado-Silva, Claudia Patricia Ornelas-García

Data type: xlsx

Explanation note: **table S1.** Topographic information and maps used in this study. **table S2.** Geomorphologic data and Nutrient Input Index values for each cave for this study.

Link: https://doi.org/10.3897/subtbiol.51.140856.suppl1

## Supplementary material 2

### $SEA_B$ analysis of Layman's metrics for the two geographic regions, Sierra de El Abra and Sierra de Guatemala, plotted as a boxplot.

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Data type: docx

Link: https://doi.org/10.3897/subtbiol.51.140856.suppl2